THE DEVELOPMENT OF PYRONEMA CONFLUENS VAR. INIGNEUM[1]

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In a preliminary note (Brown, '08) and in two subsequent papers (Brown, '10, '11) the writer has mentioned the variety named in the title as a form of *Pyronema confluens* Tul. in which the trichogyne did not fuse with the antheridium. This character and several minor ones seem, however, to distinguish it quite sharply from the ordinary form, and so it has been thought better to give it a varietal name. The one selected has reference to the conditions under which it grows.

A number of workers have quoted the writer as saying that there was no fusion of antheridium and trichogyne in *Pyronema confluens*, whereas in the note referred to (Brown, '08) it was distinctly stated that the form, there described, was probably derived from such a one as that observed by Harper ('00), in which this fusion did occur. The writer, as here described, has since observed this fusion in another form.

CULTURE OF PYRONEMA

As the generic name implies, *Pyronema confluens* usually occurs on burnt places. Harper ('00) obtained the material with which he worked in abundance, on half charred masses of leaves; but also among damp, well decayed leaves which had not been burnt. He found the plant to be extremely hydrophytic, and unable to withstand the dryness of ordinary laboratory air for even a few hours. Harper notes the differences between the above conditions and those under which Kihlman obtained what Harper believes, from the similarity of morphological structures, to be the same species. Kihlman found his material in clefts on stones and obtained successive crops by watering the stones every three or four days. Kesaroff ('08) and Seaver ('09) found that Pyronema grew abundantly on soil sterilized by heat but not on unsterilized soil. Seaver's conclusions are of special interest here, since, through his kindness, the writer has been able to repeat his experiments with the same strain and to compare it with

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the variety inigneum. Seaver found that his strain always produced abundant crops on soil or leaf-mold which had been sterilized by heating to high temperatures or by steam heat if under sufficient pressure, while he was not able in a single case to produce more than a beginning of growth on unsterilized soil. There is nothing to indicate that Seaver found this strain to be hydrophytic and the writer has found that it grew very vigorously on the outside of clay flower pots which stood in an ordinary laboratory room where the air frequently became quite dry. The differences in the conditions of sterilization and moisture under which the strains, described by Harper, Kihlman, and Seaver, were grown would seem to indicate that in Pyronema, as in *Puccinia graminis* Pers. (Eriksson, '96), there may be different physiological varieties which are entirely similar morphologically. It is, of course, uncertain as to whether these strains are permanently different or could be changed from one to another by gradually changing the environmental conditions. There is, however, nothing to indicate that the latter is the case.

In view of the experiments of Van Tieghem ('84), who found that the course of development of Pyronema was markedly influenced by the external conditions, it seems altogether likely that the growth of this plant under different physiological conditions might produce different morphological varieties and this may have been the origin of the variety inigneum.

The variety inigneum was found about the first of April, 1908, growing on a flower-pot in the botanical laboratory of the Johns Hopkins University. The ascocarps were produced to some extent on the soil inside the pot, but were much more abundant on the pot itself, where they formed large patches on both the inner and the outer surfaces. The pot on which these were found had been used for growing fern prothalli and had been watered constantly since some time during the previous October. A large number of the pots used in the laboratory had been sterilized early in October but it is uncertain as to whether or not this particular one had been. It is certain, however, that since that time, it had not been subjected to any conditions approaching sterilization.

As soon as the fungus was found, the pot on which it was growing was transferred to another room and kept watered for the next two months, during which time ascocarps continued to be produced in large numbers. They were, however, never as abundant in subsequent

crops as in the first one. This may have been due to differences in the two rooms or to some other factor; but it seems hardly likely that it was connected with conditions of sterilization, since the pot on which the fungus was growing had been kept moist for more than five months before the fungus appeared. This is more probable since Seaver ('08) found that keeping soil moist for a week destroyed the beneficial effects of sterilization. The pot was set aside early in June and allowed to dry. It was again watered about the first of the following October. Ascocarps were produced steadily during the next six weeks after which the fungus was attacked by small beetles and disappeared. It will be seen that the last ascocarps were produced more than twelve months after any possible sterilization.

During the fall of 1909 the form of *Pyronema confluens* described by Seaver ('09) was grown in the same room and on pots similar to those on which the variety inigneum had been grown the previous fall. In no case was more than a beginning of growth obtained on pots which had not been sterilized or which had been kept moist for more than a week after sterilization, while in every case in which the soil was moistened and inoculated, immediately after sterilization, ascocarps were produced in large numbers. These experiments, confirming as they do those performed by Seaver, seem to show that sterilization produced some change in the substratum which was essential for the growth of this strain. The growth of the variety inigneum seemed, however, to be quite independent of sterilization for it is hardly possible that any of the beneficial effects of sterilization should remain in a pot after twelve months, during eight of which it had been kept moist, thus allowing the growth of fungi and various micro-organisms. Although the two forms were grown at different times, the use of similar pots in the same room and at the same season of the year with such decidedly different results, in the two cases, would seem to show that the forms are quite distinct physiologically.

Development

The ascocarps which were produced on the pots were formed on the surface of a mass of hyphae which frequently grew over a felt of Penicillium. Owing to this method of growth, ascocarps of all ages could be removed from the pot with a sharp scalpel without injury. That this was the case was shown by an absence of any signs of displacement or tearing in the specimens. The material was killed in

medium chrom-acetic or Flemming's solution and stained with Heidenhain's haematoxylin or Flemming's triple stain.

For comparison with the variety inigneum the form of *Pyronema confluens* (omphalodes) described by Seaver ('09), which was grown as previously described, was prepared in the same way as the variety. In the figures of his strain, which in this discussion will be called the normal form, Seaver has figured trichogynes fused to the antheridia.

Owing to the many excellent descriptions and figures of *Pyronema confluens*, particularly the recent ones of Harper ('00) and Claussen ('12), it seems unnecessary here to more than point out the differences between the variety inigneum and the previous descriptions. The cytological features observed are very similar to those described by the writer (Brown, '11) for *Lachnea scutellata* (L.) Sacc. and present no new features.

The ascogonia of the variety inigneum are usually produced in rosettes of from fifteen to twenty. This is about twice the number in the normal form. De Bary ('84, p. 225), says that there are several, at least two or three, ascogonia in a rosette, while Harper ('00) figures three.

Many if not all of the ascogonia of a rosette of the variety inigneum are formed from the same hypha. The ascogonia are large, oval cells. Each is supported by a stalk, consisting of several cells which are smaller than the ascogonia, but larger than the other vegetative cells. The ascogonia and vegetative cells are all multinucleate. A long, slender, single-celled trichogyne is produced on the upper surface of each ascogonium. This trichogyne was found to differ from the form described by Harper, and from the normal form in lacking a beak or snout-like projection at its apex.

The antheridia are large, club-shaped, multinucleate cells and are quite similar to those figured by Harper ('00). The antheridia like the ascogonia are borne on stalks. In all cases in which the origin of an antheridium could be determined, it was found to be connected with the cell just below an ascogonium.

In the normal form, the trichogynes and antheridia become connected in the manner that has been described by Harper ('00), while it is at this point that the variety inigneum differs markedly from Harper's description. In the variety inigneum, the antheridia and ascogonia are much more independent of each other than in the normal form. Frequently an ascogonium and the antheridium connected

with its stalk are so far apart that even if the trichogyne grew straight towards the antheridium its length would not be anything like great enough to connect the two. The growth of the trichogyne appears, moreover, to be quite independent of that of the antheridium. Frequently the trichogyne grew straight out in an opposite direction from that in which the antheridium developed. At other times, a trichogyne made several coils around an antheridium, while in some cases two trichogynes were coiled around the same antheridium. In only a few cases did the trichogyne grow up over the antheridium or direct its tip against the tip of the latter as described by Harper ('00). Owing to the extremely irregular behavior of the trichogyne, the few cases in which this did occur might as well be attributed to chance variation as to any attraction between antheridium and trichogyne. In no case was the fusion between trichogyne and antheridium observed. That the irregularity just described as well as the absence of fusion were not due to displacement in handling would seem to be shown by the opposite results obtained in the normal form. The evidence from sections is very convincing. According to Harper, after the trichogyne has fused with the antheridium the two remain permanently connected. In sections the ascogonia, trichogyne and antheridia of the variety inigneum retain their normal form long after the ascogenous hyphae have grown from the ascogonia. At this stage, the ascogonia, trichogyne and antheridia are all imbedded in a firm mass of vegetative hyphae, so that it would hardly seem possible that any displacement could occur without causing considerable tearing or other disturbances which would be plainly visible in the sections. These, however, showed no evidences of tearing or displacement except in rare cases in which young ascocarps were on the edges of the mass of material.

After an ascogonium has attained its mature form, the nuclei in the trichogyne degenerate in the manner described by Harper ('00). In the process the nucleoli enlarge while the nucleus swells up and the rest of its contents become transparent. The nucleoli are usually apparent for a short time after the nuclei go to pieces, while the remains of the nuclei produce a meshlike appearance in the cytoplasm. The antheridia retain their normal appearance long after the ascogenous hyphae have grown out from the ascogonia and sometimes they appear unchanged even after the formation of the first asci. After a time, however, their nuclei degenerate in the manner described for those in

the trichogyne. After the nuclei degenerate, the antheridia become vacuolated and their contents degenerate. In the form described by Harper vacuoles appear in the antheridia after the nuclei have migrated into the trichogyne and at an earlier stage than in the variety inigneum. The behavior of the antheridia and their nuclei, as just described, would seem to be sufficient to show that the nuclei do not migrate into the trichogyne in the variety inigneum.

The development of the ascogenous hyphae is in accord with Harper's description. They grow out from the ascogonium as large branching hyphae, at the ends of which the asci are formed. The nuclei from the ascogonium migrate into these hyphae in an irregular manner and not in pairs as described by Claussen ('12). This early pairing of nuclei would not be expected in the variety inigneum for Claussen believes that each pair is composed of a nucleus derived from the ascogonium and one from the antheridium. A considerable proportion of the nuclei of the ascogonia never migrate into the ascogenous hyphae but degenerate in situ.

No fusion of nuclei, except of degenerating ones, was observed in the ascogonium or ascogenous hyphae, the only one observed being the usual one in the asci. There were, however, appearances resembling fusing nuclei. When the nucleus is preparing for division and is consequently large, the spireme or chromosomes frequently contract into a rather dense mass resembling a second nucleolus. The nucleus at this stage might readily be taken for a fusion nucleus. Also just after division, the daughter nuclei may reorganize so close to each other as to be pressed together and thus look like fusing nuclei. These phenomena have been described by the writer in detail in Lachnea (Brown, '11). The figures presented in the paper on Lachnea answer Claussen's criticism of the note on Pyronema.

The asci are formed from hook shaped hyphae at the tips of the ascogenous hyphae in the usual manner. Two nuclei divide simultaneously into four which become arranged in a uninucleate ultimate and antipenultimate cell and a binucleate penultimate one. The penultimate cell may then produce an ascus or a second hook. The ultimate and antipenultimate cells usually fuse, the nucleus of the antipenultimate one passing into the ultimate, which may then form another hook as figured by Claussen ('12) instead of degenerating as described by Harper ('00). This proliferation of the hooks, with an increase in the number of asci formed, has been described by Mc-

Cubbin ('10) for Helvella and by Brown ('10, '11) for Leotia, Lachnea and Geoglossum.

When a penultimate cell gives rise to an ascus, the two nuclei fuse, after which the fusion nucleus goes into synapsis. After synapsis a spireme emerges which contracts and divides into five chromosomes. These become arranged on the spindle and five daughter chromosomes pass to each pole. In doing so, they do not divide as in Lachnea (Brown, '11), but usually become dumb-bell shaped. In the next two divisions giving rise to eight spore nuclei, and in the first division of the spore nucleus, there are also five chromosomes. It thus appears that the same number of chromosomes, five, persists through all the divisions of the life history of the plant.

Discussion

The fusion of the antheridium and trichogyne of *Pyronema confluens* was described by the Tulasne Brothers in 1866. Their description was confirmed by Kihlmann ('83), who regarded the fusion as a proof of the sexuality of this form.

Van Tieghem ('84), grew Pyronema under various conditions, and described the course of development as being markedly influenced by the different treatments. According to this writer, under certain conditions, the chief of which is the presence of sufficient moisture, the ascogonia and antheridia are large and the trichogynes fuse with the antheridia. Under other conditions, particularly a certain amount of dryness, the antheridia and ascogonia are smaller. Under still other conditions, when the dryness passes a certain limit or the temperature falls below a certain point, the cells which usually develop into ascogonia and antheridia are smaller and more numerous than in the other two cases and there is no differentiation into ascogonia and antheridia. Since development continued in all of these cases, Van Tieghem regarded the last as an argument against the sexuality of Pyronema but said that De Bary would regard it as a case of apogamy. Harper ('00) found that, after the fusion of the antheridium and trichogyne of Pyronema, the nuclei of the antheridium migrated into the trichogyne and then into the ascogonium. According to Harper this was followed by a fusion in pairs of the nuclei in the ascogonium. Dangeard ('05) described the fusion of the antheridium and trichogyne but said that the nuclei of the antheridium did not migrate into the ascogonium. Claussen ('07) confirmed Harper's account of

the migration of the nuclei but denied the presence of a fusion of nuclei in the ascogonium. In the variety inigneum there appears to be neither a fusion of antheridium and trichogyne nor of nuclei in the ascogonium. Even though some of the above results may be due to misinterpretation it would still seem that *Pyronema confluens* may develop in a variety of ways. Some of the differences, such as those described by Van Tieghem, appear to be due to the influence of external conditions. Others, however, such as those described in this paper between the normal form and the variety inigneum may represent permanent varieties.

The absence of the fusion between the trichogyne and antheridium in some of Van Tieghem's cultures and in the variety inigneum prove that this is not necessary for the development of the ascocarp of Pyronema, while Claussen's results, together with the apparent absence of a fusion of nuclei in the ascogonia of the variety inigneum would seem to show that development can also occur without this nuclear fusion. These results, however, do not prove that a fusion of nuclei never occurs in the ascogonium. In view of the large number of pteridophytes in which apogamy has been induced, these results would not be very surprising even though it had been proved that in some cases a sexual fusion of nuclei did occur in the ascogonium. It may be said, however, that the development of Pyronema without a fusion of the antheridia and trichogynes or a fusion of nuclei in the ascogonium, together with the large number of cases among the Pezizineae in which different workers have failed to find a nuclear fusion in the ascogonium (see Brown, '11, and Claussen, '12), is not in favor of the view that such a fusion of nuclei does occur. Another argument against this fusion would seem to be afforded by the presence, in the ascogonia and in various other stages of the life history of the variety inigneum and of *Lachnea scutellata* (Brown, '11), of appearances, during the division of the nuclei, which simulate fusion quite closely. It is, of course, not safe to assume that conclusions drawn from one form will apply to another, but, as previously pointed out (Brown, '11), in view of the fact that in those Pezizineae in which divisions of the nuclei have been described in the ascogonia, these divisions show stages having the appearance of fusing nuclei, it would seem necessary to study the divisions in the ascogonia and to distinguish between true and apparent fusions before the occurrence of such a fusion can be regarded as proved, and, so far as the writer

knows, divisions have not been seen in the ascogonia of any of the Pezizineae in which a fusion of nuclei has been described at this point.

Summary

Pyronema confluens Tul. var. *inigneum* Brown agrees with the taxonomic description given by Saccardo for *Pyronema confluens* but differs from the ordinary form in that there is no fusion of antheridium and trichogyne.

The cultural conditions for the growth of the variety also appear to differ from those for the normal form in that sterilization of the substratum is unnecessary.

No fusion of nuclei was observed in the ascogonium or ascogenous hyphae. The only one observed occurred in the asci.

There are apparently five chromosomes in all of the nuclear divisions.

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